

Special Article - Gingivitis

Diagnosis of Gingivitis: State of the Art

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Gingivitis is a disease caused by accumulation of supragingival biofilm. Considering the fact that gingivitis always precedes periodontitis, the diagnosis of marginal inflammation allows monitoring the quality of at-home plaque control. Moreover, several inflammatory and/or autoimmune conditions are associated with oral mucosal manifestations. The aim of this review is to present, compare, and discuss the main methods for the diagnosis of gingivitis and autoimmune conditions associated with gingivitis. The autoimmune diseases may be diagnosed by various methods including histological examination, direct and indirect immune fluorescence microscopy, immunoblotting and quantitative immunoassay. Some gingival indices evaluate visual aspects and the presence of marginal bleeding after mechanical stimulation whereas other indices just evaluate visual aspects. In addition, some use only the extent, the length, or just the presence or absence of bleeding. Despite the fact that the collection and analysis of gingival crevicular fluid are suitable for scientific research, the diagnosis of gingivitis made by marginal bleeding is easier, faster, cheaper and, therefore, more widely applicable to routine clinical practice and epidemiological studies. The clinical diagnosis of gingivitis can thus be done by different methods. In the clinical setting, dichotomous scoring of bleeding seems to be simpler, faster, and less subjective. In the research setting, visual criteria associated with the presence of bleeding seem to more clearly detect small changes in gingival tissues, increasing the sensitivity of the selected method. The various gingival indices available share similarities and differences, but none of them is universally applied or accepted, and their selection depends on what will be evaluated.

Keywords: Inflammation; Diagnosis; Periodontics; Indice**Abbreviation**

GCF: Gingival crevicular fluid

Introduction

Gingivitis is the first sign of imbalance in the periodontal health-disease process. Plaque-induced gingivitis is caused by the accumulation of supragingival plaque around the gingival margin and is triggered between 10 and 21 days according to inter individual differences [1]. Plaque control rebalances the health-disease process and promotes the restoration of gingival health between 7 and 10 days [1-4]. Gingivitis is confined to the tissues that protect the teeth and, while not causing irreversible damage, its presence is a prerequisite for the establishment of a subgingival biofilm, which eventually leads to periodontitis [5-8]. Advanced periodontitis and dental caries are the most common causes of tooth loss in adults [9,10]. In addition, they are associated with greater impacts on quality of life for causing halitosis, pathologic tooth migration, gingival recession, bleeding, among others [11]. Besides the fact that gingivitis precedes periodontitis, the diagnosis of gingival inflammation helps the dentist to monitor sites where plaque control should be improved, i.e., the presence or absence of gingivitis is directly related to the frequency of appropriate at-home care [1]. Therefore, the diagnosis, prevention, and treatment of gingivitis are needed.

Oral lesions may be the first and occasionally the only manifestation for a number of immune-mediated diseases that affect the skin and mucosal surfaces. Autoantibodies directed

against structural compounds of the skin and oral mucosa and/or inflammatory infiltrates cause tissue damage. An accurate diagnosis can be reached by utilizing a number of diagnostic tools such as direct immunofluorescence microscopy of a perilesional biopsy and serological testing for circulating autoantibodies in conjunction with histopathological analysis. An early and precise diagnosis of autoimmune and inflammatory diseases with oral involvement is a prerequisite for their effective treatment. That being considered, the present review aims to present and discuss the different methods for the diagnosis of gingivitis described in the literature.

Literature Review

Epidemiological studies have shown a high prevalence of gingivitis and periodontitis in the general population [12, 13]. Among periodontal diseases, gingivitis is the most prevalent one, affecting almost 100% of individuals [14]. High rates of supragingival biofilm accumulation have also been observed, denoting failure in oral hygiene self-care, especially in the cleaning of proximal surfaces [15,16]. Consequently, gingivitis is quite frequent at these sites [17]. Visual signs (redness, swelling, and change in texture) and/or presence of marginal bleeding have been included as components of different indices used for the diagnosis of gingivitis [18-31]. Muhlemann & Son (1971) reported that a gingival index should be able to detect the earliest sign of gingivitis. However, there is still no consensus in the literature about the chronology of visual and inflammatory events in the pathophysiological course of gingivitis.

Table 1: The main features of the indices used to diagnosis of gingivitis.

Index	Author(s) (Year)	Instrument/Bleeding time (seconds)	Scores	Evaluated sites
Papillary Marginal Attachment (PMA)	Schour&Massler (1947)	Only visual/Not applicable	0: without gingivitis in any area of the mouth 1: mild gingivitis - inflammation located in the papilla in 1 to 3 of the 6 lower anterior teeth 2: moderate gingivitis - extension of inflammation to the gingival margin in more than 3 regions or teeth. Redness and glazing are increased in intensity 3: Severe gingivitis – extension of the inflammation to the attached gingiva. Redness, swelling, loss of dotted and tone. Spontaneous bleeding is usually present 4: very severe gingivitis - very severe generalized periodontitis.	Buccal region of all teeth; papillary, marginal, and attached gingivae evaluated separately
Gingival Index (GI)	Löe (1967)	Probe*	0: normal gingiva 1: mild inflammation - slight color change and slight edema. No bleeding on probing 2: Moderate inflammation - redness, swelling, and glazing. Bleeding on probing 3: severe inflammation - marked redness and swelling. Ulceration. Tendency to spontaneous bleeding.	Buccal, distobuccal, mesiobuccal, and lingual regions of all teeth
Sulcus Bleeding Index (SBI)	Muhlemann& Son (1971)	Probe (parallel to the long axis of the tooth)/30	0: healthy appearance of papillary and marginal gingiva, without bleeding from the sulcus 1: healthy appearance of papillary and marginal gingivae, no color change and no edema, but marginal bleeding on probing 2: bleeding on probing and color change due to inflammation. No edema 3: Bleeding on probing, color change, and slight edema 4: bleeding on probing, color change, and evident swelling or bleeding on probing and evident edema 5: bleeding on probing and spontaneous bleeding and color change, severe edema with or without ulceration.	Buccal, distobuccal, mesiobuccal, and lingual regions of all teeth
Gingival Bleeding Index (GBI)	Carter & Barnes (1974)	Unwaxed dental floss (Twice)/ Not reported; 30s is allowed for reinspection	Dichotomous (presence/absence of bleeding)	Interproximal region of all teeth except between 2 nd and 3 rd molars; areas cannot be evaluated when the position of the tooth, diastema, or other factor has a desirable interproximal relationship
Bleeding Index (BI)	Edwards (1975)	Dental tape (Twice)/15	Dichotomous (presence/absence of bleeding)	Interproximal region of all teeth
Gingival Bleeding Index (GBI)	Ainamo& Bay (1975)	Probe (3 to 4 times)/10	Dichotomous (presence/absence of bleeding)	Buccal region of all teeth
Papillary Bleeding Index (PBI)	Muhlemann (1977)	Probe*	0: no bleeding 1: only one bleeding point 2: many isolated bleeding points or only a small area of bleeding 3: interdental triangle filled with blood after probing 4: profuse bleeding when probing, blood spreads towards the marginal gingiva.	Interproximal region of all teeth
Papillary Bleeding Score (PBS)	Loesche (1979)	Wooden interdental cleaner*	0: Healthy gums, no bleeding 1: reddish gum with edema, no bleeding 2: bleeding without flow 3: bleeding with flow to marginal gingiva 4: profuse bleeding 5: severe inflammation; marked redness and edema, tendency to spontaneous bleeding.	Interproximal region of all teeth

Modified Papillary Bleeding Index (MPBI)	Barnett et al. (1980)	Probe (Once)/0-30	0: no bleeding within 30s 1: bleeding between 3 and 30s 2: bleeding within 2s 3: Immediately bleeding upon probe placement.	Mesial region of all teeth
Bleeding Time Index (BTI)	Nowicki et al. (1981)	Probe (Once or twice)/0-15	0: no bleeding within 15s of second probing 1: bleeding within 6 to 15s of second probing 2: bleeding within 11 to 15s of first probing or within 5s of second probing 3: bleeding within 10s after first probing 4: spontaneous bleeding	All teeth
Eastman Interdental Bleeding Index (EIBI)	Caton & Polson (1985)	Interdental wooden cleaner (4 times)/15	Dichotomous (presence/absence of bleeding)	Buccal in interproximal regions
Quantitative Gingival Bleeding Index (QGBI)	Garg & Kapoor (1985)	Dental brush*/30s is allowed for reinspection	0: no bleeding on brushing; bristles free of blood stains 1: slight bleeding on brushing; bristle tips stained with blood 2: moderate bleeding on brushing; about half of bristle length from tip downwards stained with blood 3: severe bleeding on brushing; entire bristle length of all bristles including brushhead covered with blood	1 score for each 6 segments: canine to canine or premolars and molars, left or right, in the upper or lower arches
Modified Gingival Index (MGI)	Lobene et al. (1986)	Only visual/Not applicable	0: no inflammation 1: mild inflammation; slight color change, slight change in texture but not in all papillary or marginal gingivae 2: mild inflammation; same criterion as in score 1 but involving all papillary unit or marginal gingiva 3: moderate inflammation; glazing, redness, swelling and/or hypertrophy of the papilla or marginal gingiva 4: severe inflammation; marked redness, swelling and/or hypertrophy of the papilla or marginal gingiva, spontaneous bleeding or ulceration	Buccal, distobuccal, mesiobuccal, and lingual regions of all teeth
Bleeding on Interdental Brushing Index (BOIB)	Hofer et al. (2011)	Interdental brush (Once)/30	Dichotomous (presence/absence of bleeding)	Interproximal region of all teeth

Diagnosis of gingivitis

Gingivitis can be diagnosed by various methods. Although histological evidence of inflammation is an accurate method to assess gingivitis, biopsies are impracticable. Therefore, a less invasive method is required [32]. The measurement of GCF has proven to play an important role in the assessment of gingivitis [33-36].

Categorical scores have been used by different indices. Such indices combine visual aspects and the presence of marginal bleeding after mechanical stimulus [26,27,29]. Other indices only evaluate visual aspects [25,31]. Also, some use only the extent of bleeding [23,28], bleeding time [19,30], or just the presence or absence of bleeding [18, 20-22, 24]. It is difficult to determine which criteria (GCF volume, visual signs, or gingival bleeding) best indicate the inflammatory condition of the gingiva given that some evidence has shown weak correlations between clinical criteria/gingival fluid and inflammatory status observed histologically [35,37-40]. Thus, comparisons between different diagnostic methods could be inaccurate.

According to Carter & Barnes (1974), a good index for evaluating gingivitis must have well-established validity in order to assess what actually needs to be assessed and enough sensitivity to detect slight changes. Moreover, its reproducibility by the same or different examiners is also crucial. Finally, an index should be simple to use, require few tools, and be as free as possible from subjective interpretation.

Several methods for stimulation of marginal bleeding have been used: periodontal probe [18,19,26,28-30], wooden interdental cleaner [21,27], dental floss [20], dental tape [22], toothbrush [23], and interdental brush [24]. Table 1 shows the main features of the indices used to date.

GCF

GCF results from the interaction between the bacterial biofilm attached to the tooth surface and periodontal tissue cells [41]. It is a complex mixture of substances derived from blood serum, leukocytes, structural cells of the periodontium, and oral microorganisms.

Thus, GCF analysis is a noninvasive measure that assesses the pathophysiological state of the periodontium at a specific site [42].

GCF is constantly secreted [43-45]. Löe& Holm-Pedersen (1965) reported that GCF flow is proportional to the severity of inflammation, thereby highlighting its importance as an assessment tool. They concluded that, in order to obtain valid measurements of the fluid, paper strips should be positioned at the entrance (extrasulcular method) rather than within the gingival sulcus (intrasulcular method proposed by Brill & Krasse in 1958) until some resistance is felt. These methodological differences probably affect the results, since even a gentle insertion into the gingival sulcus causes sufficient damage, changing the permeability of the epithelium and, consequently, increasing the amount of gingival fluid [46].

A low GCF flow is associated with healthy tissue while a high GCF flow indicates inflamed tissues [33,35]. Visual signs of inflammation have been associated with an increased GCF flow [35,36,45,47], and so has gingival bleeding [30,35,36,48-50]. A higher GCF flow is observed in multirrooted teeth when compared to single-rooted ones, probably due to the difficulty faced by individuals in performing oral hygiene on these teeth or to the anatomy of molars (greater interproximal root surface, possibility of larger root irregularities, and abundant vascularization) [34].

Several methods have been developed for GCF collection, such as the gingival washing method [51], the use of micro capillary tubules or micropipettes [52], and absorbent filter paper strip collection [44]. The downside of the washing method is that it does not provide information on the volume of collected fluid and, although the capillary tubing method measures different amounts of fluid, it requires a long time (around 30 minutes per site) for an accurate collection of small volumes [53]. Moreover, unlike the absorbent filter paper strip method, which is fast, easy to use, minimally invasive and has traditionally been the method of choice [53,54], the use of capillary tubes can cause trauma and affect the measurement of the volume and components of the collected fluid.

Different types of absorbent strips are available: Durapore, Millipore [55], Whatman chromatography [56], and absorbent filter paper strip [57]; however, none of them have had their validity tested with Periopaper®. Periopaper® is a filter paper strip widely recognized as a method of choice for GCF collection via absorption [54,58].

Due to the importance of quantifying GCF volume, a number of methods have been described for measuring it via absorption: colorimetry, weighing, and use of an electronic apparatus (Periotron®). Colorimetry is a valid method that uses ninhydrin or fluorescein to indicate areas of absorption; however, the stains obtained by this technique and by weighing do not allow the analysis of GCF components. More recently, the introduction of an electronic device known as Periotron® has allowed a more accurate determination of GCF volume, enabling subsequent laboratory research into sample composition [53]. The equipment measures the electrical capacitance of the filter paper strip [59]. There are three Periotron® models (600, 6000, and 8000) and all have shown accuracy in GCF volume measurement [53]. Periotron® 8000 (Ora Flow Inc., Amityville, NY, USA) quantifies the amount of GCF or saliva collected with filter paper strips and, by using a computer program, it converts the data

into a unit of volume [60]. It is recommended that the GCF collected on Periopaper® strips be immediately transferred (within 0-2s) to Periotron® to prevent the material from evaporating [61,62].

Other operational and technical aspects, such as collection time; contamination of GCF samples by blood, saliva, and plaque; and air temperature and humidity, can interfere with measurement accuracy [34,53,60,63,64]. Both knowledge and control of these aspects ensure that the observed results will actually reflect the condition of the investigated tissue. Previous studies on Periotron® have suggested that the filter paper strips should remain in place for 5s [53]. However, alternative approaches have been developed to increase the GCF volume available for subsequent laboratory analysis [53]. One of them consists in leaving the strip at the entrance of the gingival sulcus for 30s [65] or 3 min [36,44]. A study with gingivitis patients compared these two collection times and found no difference in fluid volume proportional to the increase in measurement time [66]. Based on the results, the authors recommend restricting the collection time to 30s, thus safely determining the extent of gingival infection. Nevertheless, the problem with a long collection time is that the nature of the fluid samples may change, especially regarding protein concentration [67].

The volume and flow rate of GCF are indicators of vascular permeability changes at the early stages of inflammation [68]. Then, the standard clinical measurements used to determine gingival inflammation may be less sensitive than GCF results, showing better diagnostic accuracy of this method at earlier stages of gingivitis [41,53]. However, although GCF collection and analysis are suitable for scientific research, the diagnosis of gingivitis made by marginal bleeding is easier, faster, cheaper and, therefore, more widely applicable to routine clinical practice and epidemiological studies.

Visual criteria versus marginal bleeding

Some gingival indices have been based on clinical features of inflammation, with some components evaluated noninvasively by visual examination (color, texture, shape, spontaneous bleeding), and inflammatory components measured invasively after some stimulus. The visual signs of gingival inflammation include redness of the gingival margin, which becomes evident from vasodilation, and increase in the number of vascular units in the sub epithelial connective tissue [69], since edema and the smooth texture of the free gingiva indicate loss of fibrous connective tissue and extravasation of inflammatory cells into the extracellular matrix. Bleeding after a stimulus is due to microulcerations in the sulcular epithelium [70]. This parameter, for being objective and easy to use, has often been considered in the evaluation of the gum [71-73].

A diagnostic index for gingival conditions should be simple and quick to use, with clear and comprehensible criteria, and should also be sensitive to identify variations at different stages of the disease [25]. In this sense, visual criteria (color, swelling, texture) hinder clinical and epidemiological application as they are time-consuming, do not allow easy assessment of proximal regions (especially of posterior teeth), are subjective, and are not determined only by inflammatory components, but also by variations in the intensity of melanogenesis and in the degree of keratinization and vascularity [70].

Given the limitations of visual aspects in the diagnosis of gingival changes, the presence or absence of bleeding on probing [18] is more

universally applicable in clinical and epidemiological studies and in clinical practice [20,29]. Although gingival bleeding on probing is not a good diagnostic indicator of clinical attachment loss, its absence is an excellent negative predictive sign of future insertion loss [74].

Some authors have shown that, even in the absence of visual changes, a significant percentage of sites show marginal bleeding [29,75,76], which means that the presence of bleeding is a sign that precedes visual changes [18,20,29,75-77]. Other authors have noted that changes in color and contour precede marginal bleeding at the early stages of gingivitis, [70,78]. This discrepancy may be due to the subjectivity of visual inspection and to the differences in the techniques used to evaluate bleeding [70], possibly increasing the number of false positive results in consequence of trauma after mechanical stimulation.

Periodontal probe versus dental floss/tape

Variations in probing depth and angulation may interfere with the results by stimulating bleeding in deeper regions of the pocket or by causing injury, hindering the diagnostic value of marginal bleeding on probing [79,80].

There is evidence that gingival inflammation in the proximal region likely arises in the center of the papilla [21,81,82], an area that is not often thoroughly assessed by the probe at sites without attachment loss and with an established point of contact. Thus, it appears that a marginal probe for the diagnosis of gingival conditions has a somewhat limited use in proximal regions. Therefore, the use of dental floss/tape as a diagnostic tool may be advantageous in the proximal region as it allows contact along the full length of the papilla.

Dental floss/tape versus wooden interdental cleaner versus interdental brush

Gingival indices that use wooden interdental cleaner for detecting proximal gingivitis [21,27] can cause trauma to the tissue due to the shape and rigidity of these devices and should thus be used with caution. However, the index proposed by Hofer et al. (2011), which relies upon the insertion of an interdental brush into the vestibular region below the point of contact, cannot be used when the papilla fills the interproximal region. Among the four devices assessed, dental flosses and tapes seem to be the most suitable to detect proximal gingivitis, possibly because they do not cause trauma to the gingival tissue and can be inserted into the proximal sites with or without the presence of papillae.

Gingivitis associated with inflammatory and autoimmune diseases

A group of autoimmune diseases is characterized by autoantibodies against epithelial adhesion structures and/or tissue-tropic lymphocytes driving inflammatory processes resulting in specific pathology at the mucosal surfaces and the skin [83]. The most frequent site of mucosal involvement in autoimmune diseases is the oral cavity. Broadly, these diseases include conditions affecting the cell-cell adhesion causing intra-epithelial blistering and those where autoantibodies or infiltration lymphocytes cause a loss of cell-matrix adhesion or interface inflammation [84]. Several inflammatory and/or autoimmune conditions such as chronic ulcerative stomatitis, lichen planus, mucous membrane pemphigoid, pemphigus vulgaris, erythema multiforme, plasma cell gingivitis and graft-versus-host

disease are associated with oral mucosal manifestations, including "desquamative gingivitis" [85]. This term was introduced to describe the presence of erythema, localized or generalized desquamation and/or erosion on the buccal aspect of attached gingiva mainly of the anterior teeth. In some cases, marginal gingiva may also be affected. Gingival desquamation has a subacute or chronic onset in the majority of cases, with variable degrees of extension and distribution [85].

Studies show that oral lichen planus is the most common immune-mediated disorder affecting the oral cavity, followed by pemphigus vulgaris and mucous membrane pemphigoid [86,87]. Moreover, oral mucosa can be the first affected mucosal surface in many of these conditions, a fact that emphasizes the need for better understanding of clinical features and diagnostic tools for autoimmune diseases among practitioners. Precise and early diagnosis greatly facilitates timely, effective and specific treatment [86]. The definitive, accurate diagnosis of autoimmune diseases requires the detection of immunoreactant deposits in the tissues and the circulating autoantibodies by direct and indirect immunofluorescence microscopy, respectively. Direct immunofluorescence microscopy helps to detect molecules such as immunoglobulins and complement within biopsy specimens [88]. Selection of the site for the biopsy specimen is important. Direct IF microscopy is performed on non-bullous or non-eroded skin or mucosa (i.e. erythematous or normal appearing tissue adjacent to blisters or erosions), because immune deposits may be degraded in the area where the dermal-epidermal separation occurs, leading to false negative results. False negative results may also occur as a result of improper handling or faulty preservation of the biopsy, which must be frozen immediately and stored at temperatures below -70°C or placed in a saline or a special Michel's medium for transport for no longer than 48 hours for subsequent immunofluorescence testing [89].

Indirect immunofluorescence microscopy is a test in which patient's serum is examined for the presence of circulating autoantibodies to a defined antigen. This test allows the differentiation between serum autoantibodies that bind to the roof and those that stain the floor of the artificial split reflecting the molecular difference in autoantibody specificity [89].

A number of other immunoassays, including Enzyme-Linked Immunosorbent Assay (ELISA), immunoblot or immunoprecipitation are available to facilitate the characterization of the molecular specificity of autoantibodies. Of these techniques, the ELISA is most commonly used. With the identification of target antigens and advancement of molecular biology and recombinant technology, antigens have been produced in bacteria and eukaryotic cells [88]. These recombinant, cell derived forms of the target antigens have been utilized in the development of sensitive and specific ELISA kits for detection of circulating autoantibodies. ELISA using recombinant antigens has several advantages over indirect immunofluorescence techniques on tissue sections. It provides information on the molecular specificity of autoantibodies, it is easy to perform and readily amenable to standardization, and, importantly gives quantitative results. Therefore, these are exquisite parameters for monitoring diseases, in which levels of serum autoantibodies have been shown to correlate with disease activity. Several commercially available ELISA kits are now used for the diagnosis and monitoring of immune-mediated diseases [90].

While the autoimmune disease may be suspected based on clinical manifestations, demonstration of tissue-bound and circulating autoantibodies, or lymphocytic infiltrates, by various methods including histological examination, direct and indirect immunofluorescence microscopy, immunoblotting and quantitative immunoassay is a prerequisite for definitive diagnosis.

Concluding Remarks

Gingivitis can be clinically diagnosed by different methods. In the clinical setting, dichotomous scoring of bleeding seems to be simpler, faster, and less subjective. Moreover, the absence of gingival bleeding on probing is desirable, indicating low risk of future clinical attachment loss. In the research setting, visual criteria associated with the presence of bleeding seem to more clearly detect small changes in gingival tissues, increasing the sensitivity of the selected method.

Moreover, given the frequency of oral involvement and the fact that oral mucosa is the initially affected site in many cases, the informed practitioner should be well acquainted with diagnostic and therapeutic aspects of autoimmune dermatosis with oral involvement.

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